Clinical Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Case Report

Spurious testosterone laboratory results in a patient taking synthetic alkaline phosphatase (asfotase alfa)

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ARTICLE INFO

Keywords:
Testosterone
Alkaline Phosphatase
asfotase alfa
Juvenile hypophosphatasia

ABSTRACT

Objectives: We report a case of discordant total and free testosterone values in a patient with hypogonadism and juvenile hypophosphatasia after he initiated treatment with asfotase alfa, recombinant tissue non-specific alkaline phosphatase.
Methods: Total testosterone was evaluated using immunoassay pre and post initiation of therapy with asfotase alfa, and free testosterone was evaluated using radioimmunoassay and LC-MS/MS while on asfotase alfa therapy.
Results: Total testosterone measured by immunoassay was normal prior to therapy with asfotase alfa, and was low post initiation of therapy. During the same time frame, free testosterone measured using RAI and total testosterone measured using LC-MS/MS were normal on asfotase alfa therapy. This suggests assay interference with the total testosterone immunoassay.
Conclusion: When laboratory results are discordant or do not match the clinical impression, the possibility of assay interference should be considered. Alternative laboratory methods free of the interference should be selected to evaluate these patients.
Human genes discussed in the paper: ALPL gene, Approved name: Alkaline phosphatase, liver/bone/kidney, Synonym: Tissue non-specific alkaline phosphatase (TNSAP).

1. Introduction

Hypophosphatasia (HPP) is a rare inherited disease characterized by decreased activity of tissue non-specific alkaline phosphatase (TNSALP) [1]. The severity and age of onset of HPP is highly variable. It is generally categorized into six forms based on presentation and age of onset as: perinatal lethal, perinatal benign, infantile, juvenile, adult, and odontohypophosphatasia [1]. Asfotase alfa, a pharmacologic recombinant TNSAP enzyme replacement therapy, was FDA approved in the United States in October 2015 as the first therapy approved for the treatment of patients with HPP [2]. It is a soluble glycoprotein that replaces the function of endogenous alkaline phosphatase, and incorporates the TNSALP active site into the structure of the recombinant agent. Whyte et al. showed that asfotase alfa therapy resulted in markedly elevated levels of TNSAP [3]. When it was initially approved, there were no reports of other laboratory abnormalities or interference from the drug. However, it has been reported that elevated endogenous levels of TNSALP > 1000 U/L can be associated with falsely elevated cardiac Troponin I and β-hCG levels measured with the Beckman Coulter DxI 800 AccuTnI + 3 and Beckman Coulter DxI 800 total hCG assays respectively, which use alkaline phosphatase (ALP) for signal amplification [4]. Immunoassays using ALP-labeled tracers are commonly used for measurement of various analytes in clinical laboratories. Therefore, in patients with HPP who are treated with asfotase alfa, the structural similarities and possible similar association rate constants between the asfotase alfa and the reagents used in the assay have the potential to lead to spurious results for certain lab tests.

We present a novel case of falsely low serum total testosterone, with concurrent normal free testosterone in a patient with HPP after initiation of therapy with asfotase alfa.

We will discuss the design of various free and total testosterone assays, correlate the laboratory and clinical findings with initiation of therapy with asfotase alfa, review the molecular structure of asfotase alfa, and propose potential mechanisms of analytical interference of this

Abbreviations: ALP, Alkaline phosphatase; HPP, Hypophosphatasia; LC-MS/MS, Liquid chromatography–mass spectrometry/mass spectrometry; RIA, Radioimmunoassay; SHBG, Sex hormone binding globulin; TNSALP, Tissue non-specific alkaline phosphatase; TST, Testosterone

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https://doi.org/10.1016/j.clinbiochem.2018.04.024
Received 26 October 2017; Received in revised form 25 April 2018; Accepted 26 April 2018

drug with the total testosterone assay. To our knowledge this is the first report regarding interference of asfotase alfa with the total testosterone assay.

2. Case Description

We report the case of a 33-year-old male who presented in his late teens with multiple non-healing extremity fractures, was ultimately noted to have low TNSAP, and bone biopsy of the iliac crest revealed adynamic bone disease. Genetic testing showed a heterozygous mutation in the ALPL (c.406C > T; p.R136C) gene consistent with HPP. Subsequently, he was also diagnosed with secondary hypogonadism. Testosterone was evaluated because of fatigue and was low, while FSH and LH were inappropriately normal, consistent with secondary hypogonadism. MRI of the pituitary was normal. He was successfully treated with testosterone gel and was consistently compliant with therapy. His total and free testosterone levels returned to normal, and symptomatically his energy improved. He was maintained on a stable dose of testosterone for many years and consistently had normal free and total testosterone levels (Table1).

Once asfotase alfa was FDA approved, he initiated therapy for his HPP.

One month after asfotase alfa was initiated, 8 am serum total testosterone was undetectable at < 0.3 nmol/L (Beckman Coulter DXI 800), (reference range: 6-271 nmol/L). This was a significant reduction from his previous check 10 months prior, when it was normal at 13 nmol/L (Table 1). Free testosterone was evaluated via radioimmunoassay (RIA) (DSL-4900 Active Free Testosterone Coated-Tube Radioimmunoassay kit, Beckman Coulter and ^{125}I labeled Testosterone Tracer) and was still within the normal range at 26.4 pmol/L (reference range: 17.7–144 pmol/L). Despite his low total testosterone, he clinically felt much better after starting the asfotase alfa, and did not have any signs or symptoms suggestive of hypogonadism.

Additionally, evaluation for the presence of interfering HAMA antibodies was negative. Given the proximity of the abnormal total testosterone with the initiation of asfotase alfa, and a concurrently normal free testosterone, we suspected laboratory interference and a spuriously low total testosterone value, so continued the same dose of testosterone. At his next follow up five months later, his total testosterone was again undetectable (Beckman Coulter DXI 800). Repeat total and free testosterone showed similar results: total testosterone < 0.3 nmol/L (Beckman Coulter DXI 800), free testosterone normal at 23.25 pmol/L (RIA). Since the total and free testosterone were discordant, total testosterone was also evaluated with liquid chromatography- mass spectrometry (LC-MS/MS), and was in the normal range at 16.4 nmol/L (reference range: 8.32-32.96 nmol/L). LC-MS/MS was performed using the Applied Biosystem-Sciex API-3000 triple quadruple mass spectrometer. This used the deuterated stable isotope (d3-testosterone) after protein precipitation with acetonitrile and extraction utilizing high-throughput liquid chromatography, followed by conventional liquid chromatography; analysis was done with a heated nebulizer ion source.

After 22 months of therapy, the patient felt he was no longer responding to the asfotase alfa, so it was discontinued. TNSAP normalized off asfotase alfa therapy and once TNSAP levels were normal, repeat total testosterone (Beckman DXI immunoassay) was again normal at 7.42 nmol/L (reference range 6.07-27.1 nmol/L). The dose of testosterone gel did not change throughout this time frame.

3. Discussion

This case is unique because the patient has both HPP and secondary hypogonadism, which necessitated routine serum testosterone measurements, which is not common for most patients with HPP on asfotase alfa. Prior to treatment with asfotase alfa, both total and free testosterone levels were normal on testosterone therapy. One month after initiation of asfotase alfa treatment, free and total testosterone labs were discordant despite clinical improvement and no change to his medications or other health history. This prompted further investigation. The total testosterone was surprisingly low when measured by an immunoassay that uses ALP as a conjugate. Therefore, total testosterone was evaluated via ED-LC-MS/MS, which is the gold standard for testosterone evaluation. Both, the total testosterone measured via ED-LC-MS/MS and the free testosterone measured by RIA, were normal and neither one of these assays are using ALP labels. The discrepancy between the concentration of total testosterone measured via immunoassay and that measured via ED-LC-MS/MS (Table 1) suggested an interference with the total testosterone immunoassay. At the time of these laboratory evaluations, it was not known that asfotase alfa had the potential to interfere with immunoassays that used ALP as a conjugate in the assay.

Interestingly, during the same time frame, the patient was admitted to the hospital for chest pain and cardiac troponin I was elevated on multiple occasions with negative cardiac workup, including a cardiac catheterization. After asfotase alfa therapy was discontinued, he had another episode of chest pain and cardiac troponin I was normal. Herman et al. has previously reported falsely elevated cardiac troponin I values when endogenous TNSAP was > 1000 U/L when using the Beckman Coulter DXI-800 assay which uses ALP as a conjugate in the assay [4]. The authors also tested cardiac troponin I using Siemen's Vista Luminescent oxygen Channeling Immunoassay which does not require ALP, and level was normal, suggesting that elevated TNSAP interferes only with the immunoassays that use ALP as the conjugate [4].

This case underscores the importance of understanding the methodology of an assay when ordering clinical tests. Both free and total testosterone can be measured using various methodologies including competitive immunoassays, RIA, LC-MS/MS post equilibrium dialysis or ultrafiltration. There are, however, limitations to each method. Immunoassays are prone to multiple interferences, either analyte-independent (e.g. lipemia) or analyte-dependent (e.g. cross-reaction with antibodies, such as heterophile antibodies, autoantibodies, rheumatoid factor, complement, lysozyme, paraproteins, medication, metabolites, etc.) [5]. Cross-reaction can lead to spurious laboratory results (high and low) when endogenous molecules or medications present in a patient's blood sample have common cross-reactive epitopes with similar structures to the measured analyte. Falsely low laboratory results have been reported with competitive immunoassays [6]. The cross-reactant competes with the analyte of interest for binding to the antibody sites with similar association rate constants. If the dissociation rate for the cross-reactant is greater than that of the primary ligand, more unoccupied sites would become available to bind the cross-reactant. The outcome is reduced recovery of the analyte of interest [6]. Inadequate

Table 1
Laboratory results prior to and after administration of asfotase alfa.

Time in relation to starting asfotase alfa (+ or − months)	−24	−18	−10	−12	+1	+5	+6	+12	+13	+18	+22 off asfotase alfa
ALP (Beckman AU 5800) (ref. range 0.54-1.54 μkat/L)	0.5	0.5	0.4	0.6	91.5	124		137	80	116.3	1.4
Total testosterone (Beckman DXI 800) (ref range: (6-27.1 nmol/L)	8.9	10.5	10.1	13	< 0.3	< 0.3	< 0.3	3.4	3.9	< 0.3	7.4
Free Testosterone (RIA) (ref. range: 17.7-144 pmol/L)		52.7	46.2	48.6	26.4		23.3	82.2	90.2		
Total Testosterone (LC-MS/MS) (ref. range 8.3-33 pmol/L)								16.4	12.5		